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Jahnula morakotii sp. nov. and *J. appendiculata* from a peat swamp in Thailand

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Abstract — During a long-term study of wood colonization by freshwater fungi in the Sirindhorn peat swamp forest in the south of Thailand, two interesting *Jahnula* species were encountered. *Jahnula appendiculata* occurred commonly on eight species of timber, while *J. morakotii* occurred only once. *Jahnula morakotii* differs from all other *Jahnula* species in having the smallest ascospores with bipolar cellular appendages and lacking a sheath. The morphology of *J. morakotii* is illustrated and compared with other species in the genus.

Keywords — *Ascomycota*, colonization of wood, systematics

Introduction

During a long-term colonization study by freshwater fungi, of wood submerged in the Sirindhorn peat swamp forest, Narathiwat, in the south of Thailand, two *Jahnula* species were found.. After several years of wood exposure, *Jahnula appendiculata* and *J. morakotii* were encountered. *Jahnula appendiculata* was found several times on test blocks of seven timber species after two to three years of submergence, while *J. morakotii*, was found only once on one timber species after the wood had been exposed for two years.

All thirteen *Jahnula* species that have been described occur in freshwater habitats and mostly from tropical regions (Hyde 1992, Hyde & Wong 1999, Pang et al. 2002, Pinruan et al. 2002, Raja & Shearer 2006, Raja et al. 2009). Thus far, *J. appendiculata* and *J. morakotii* are known only from the Sirindhorn peat swamp forest in Thailand (Pinruan et al. 2002 and this study) and may be restricted to this unique habitat (water pH 5.8–6.2, with a river system running through this acidic peat bog). *Jahnula appendiculata* was first described on a natural submerged palm trunk (Pinruan et al. 2002), while *J. morakotii* was collected on a single test block of *Azadirachta indica* var. *siamensis*. The

characteristic features of the new species include: globose to subglobose, always stalked, superficial ascomata, pseudoparaphysate hamathecium, bitunicate, fissitunicate asci, and brown, uniseptate ascospores with bipolar cellular appendages. These traits are congruent with taxa in the *Jahnulales,* especially the genus *Jahnula*. However, this fungus could not be assigned to any species currently included in *Jahnula* and is therefore described as new.

Materials and methods

Nine timber species (*Azadirachta indica* var. *siamensis* Valeton, *Erythrophleum teysmannii* Craib, *Melaleuca cajuputi* Powell, *Shorea obtusa* Wall., *S. roxburghii* G. Don, *S. siamensis* Miq., *Wrightia tomentosa* Roem. & Schult., *Xylia xylocarpa* (Roxb.) W. Theob., *Zollingeria dongnaiensis* Pierre) were submerged in the Sirindhorn peat swamp forest in Narathiwat Province, Thailand on 12 March 2001 in order to follow their colonization by freshwater fungi over a 10-year period. Twelve sets of test blocks (15 × 2.5 × 2.5 cm^3, 5 blocks per set for each timber species), free of preservative, were threaded on a nylon rope and autoclaved 3 times before submergence in the Sirindhorn peat swamp forest in Narathiwat.

Nine sets of test blocks (one of each timber species) were recovered at 1 and 6 months, and 1, 2 and 3 years and returned to the laboratory in a clean polystyrene foam box. Test blocks were washed with stream water to remove silt and mud from the surface. Each set of test blocks was separated and single blocks were placed in pre-sterilized plastic boxes with moist tissue papers layered on the bottom. Test blocks were incubated at 20°C in a cabinet with cool white fluorescent light. Test blocks were examined for sporulating fungi after one week, and 1, 2 and 3 months following removal from the river. Assessment procedures were as described by Sivichai et al. (2002).

Material was examined using a stereomicroscope and fungi isolated and identified. Preparations were mounted in lactophenol-cotton blue, and sealed with polyvinyl alcohol. Single-ascospore isolations were made and grown on Corn Meal Agar (CMA, Difco?). Ascospores were spread over the agar surface with a flame-sterilized inoculation loop dipped in 0.05% (w/v) Triton X-100. Plates were incubated at 20°C in a cabinet with cool, white fluorescent light and examined with a microscope each day for signs of germination. Six to eight germinated ascospores were transferred to new plates and incubated in the same cabinet. Dried specimens are deposited in the BIOTEC Bangkok Herbarium (BBH #27681).

Taxonomic description

Jahnula morakotii Sivichai & Boonyuen, **sp. nov.** FIGS. 1–11

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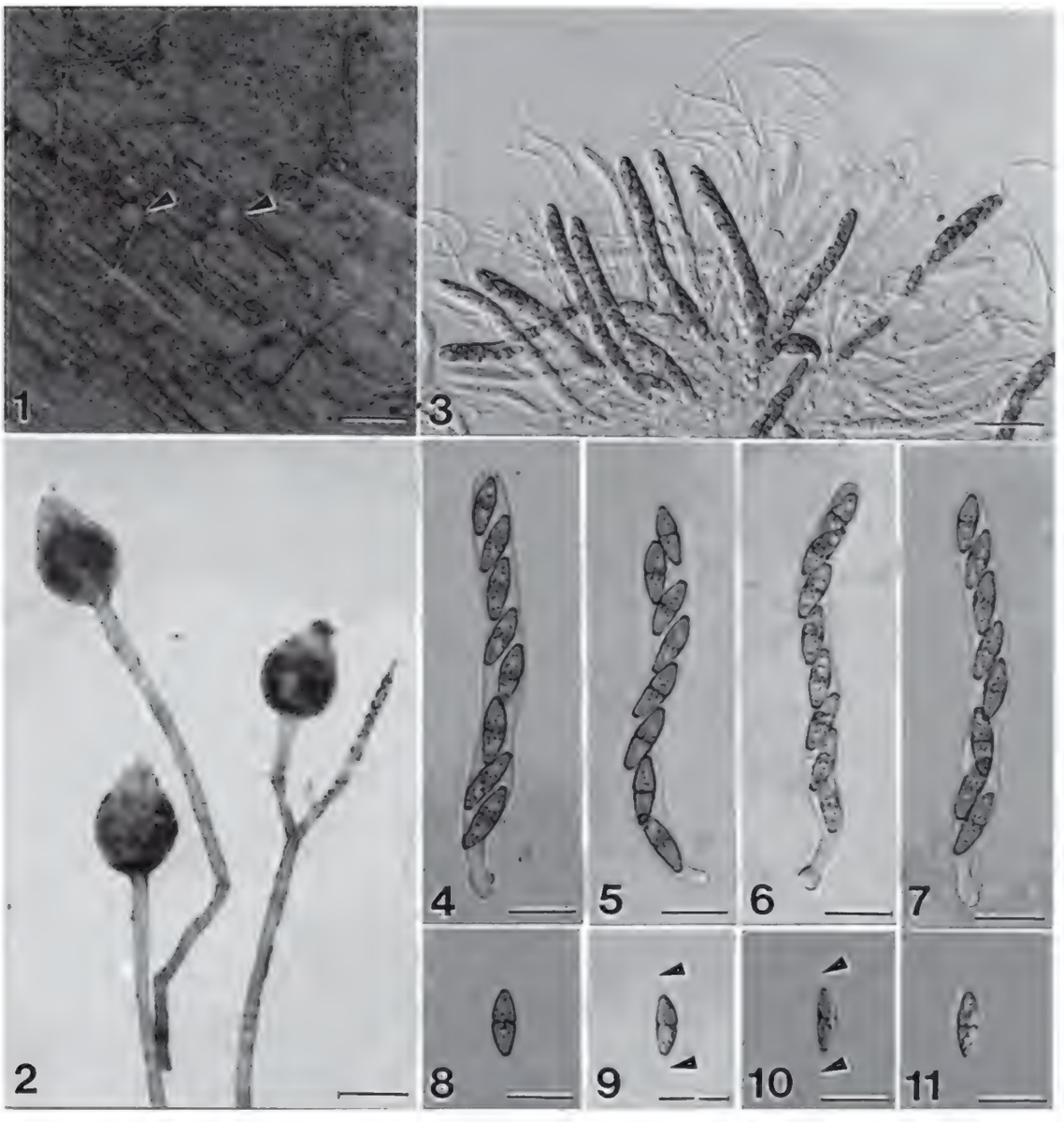
Ascomata 100–180 µm diametro, globosa ad subglobosa, gregaria, superficialia cum caule vel sessilia. Pseudoparaphyses septatae, hyalinae 1.5–2 µm lata, ca. 150 µm longis. Asci 107.5–120 × 9–11.5 µm, octospori, cylindrici, pedicellati, bitunicati, fissitunicati, camera oculari et annulo tenui instructi. Ascosporae 17.5–20 × 5–6.5 µm, fusiformae?, brunneae, guttulatae uniseriatae vel biseriatae, uniseptatae, constrictae, rectae vel aliquantum curvatae, duobus appendicibus cellularibus terminalibus longis hyalinis praeditae.



Figs. 1–11. *Jahnula morakotii.* (Holotype SS2447). 1. Superficial ascomata on test block (arrowed). 2. Ascomata with long and septate stalks. 3. Squash mount of asci and pseudoparaphyses. 4–7. Cylindrical asci with pedicels. 8–11. Ascospores with bipolar appendages (arrowed).
Measure bars for Fig. 1 = 200 µm. Fig. 2 = 100 µm. Fig. 3 = 30 µm. Figs. 4–11 = 20 µm.

Holotype: Thailand. Narathiwat: Sirindhorn peat swamp forest on submerged wood test block (*Azadirachta indica*), 10 March 2003 by Somsak Sivichai & Nattawut Boonyuen, BIOTEC SS2447.

Etymology: "*morakotii*" in honor of Professor Morakot Tanticharoen, the past director of BIOTEC: National Center for Genetic Engineering and Biotechnology, who supports our Mycology Laboratory in Thailand.

Ascomata 100–180 µm diam, globose to subglobose, superficial with septate stalk, 18–30 µm wide, or sessile (Figs. 1–2). Peridial wall of large, thin-walled cells. Pseudoparaphyses septate, hyaline, 1.5–2 µm wide, up to 150 µm in length

(Fig. 3). Asci 107.5–120 × 9–11.5 µm (mean = 116 × 11 µm, n = 50), 8-spored, cylindrical, pedicellate, bitunicate, fissitunicate, with a shallow ocular chamber and faint ring (Figs. 3–7). Ascospores 17.5–20 × 5–6.5 µm (mean=19 × 6 µm, n = 50), fusiform, brown, multi-guttulate, uniseriate or biseriate, slightly constricted at the septa, straight to curved with cellular bipolar hyaline apical appendages.

Habitat: Saprobic on submerged wood test block (*Azadirachta indica*) in peat swamp forest.

Geographical distribution: Thailand.

Comments: *Jahnula morakotii* was collected only once on an *Azadirachta indica* test block, and it can be considered a rare fungus. *Jahnula morakotii* differs from all *Jahnula* species in having the smallest ascospores among all described species (17.5–20 × 5–6.5 µm). Species most similar in ascospore size to *J. morakotii* are *J. bipileata* Raja & Shearer (25–30 × 9–10 µm) and *J. australiensis* K.D. Hyde (19–30 × 6–8 µm), however, they lack the bipolar appendages of *J. morakotii* (Raja & Shearer 2006). *Jahnula appendiculata* is the only other species with bipolar appendages but the ascospores of this species are longer and wider (45–52.5 × 22.5–27.5 µm) than those of *J. morakotii* (17.5–20 × 5–6.5 µm). In addition, ascospores of *J. appendiculata* have a thick sheath that is absent in *J. morakotii* (Pinruan et al. 2002).

Jahnula appendiculata Pinruan, K.D. Hyde & E.B.G. Jones, Sydowia 54(2): 243. 2002. Figs. 12–19

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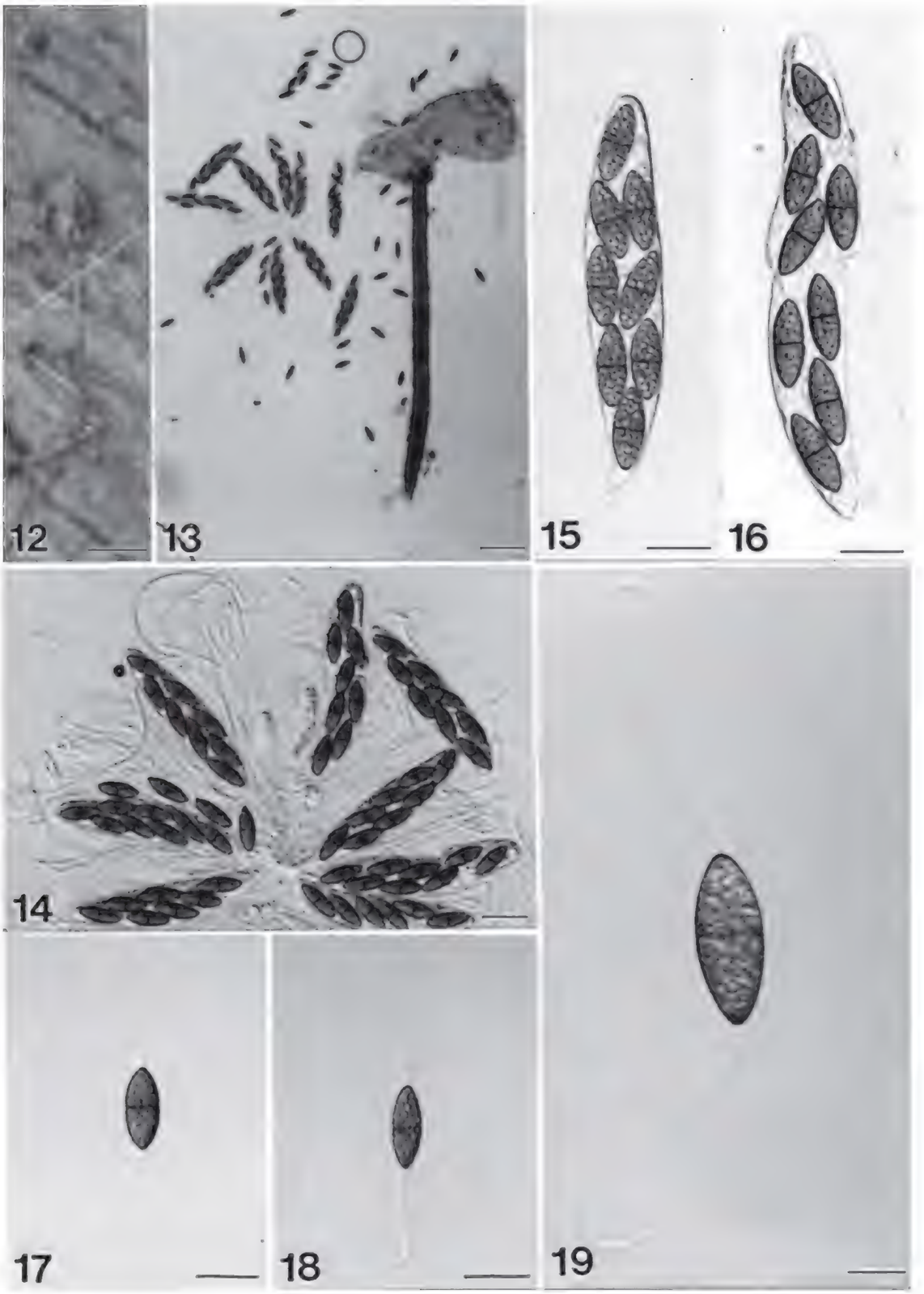
Ascomata 280–350 µm in diam, pale brown, globose to subglobose, semi-immersed, becoming erumpent, but with the base remaining immersed, or superficial with stalk attached to the base (Fig. 12), stalk up to 2 mm long and 65 µm wide, brown (Fig. 13). Pseudoparaphyses septate, hyaline, 2–3 µm wide and up to 500 µm long, filamentous, septate, unbranching (Fig. 14).

Asci 320–450 × 38–45 µm (mean = 400 × 42 µm, n = 50) (Figs. 14–16), 8-spored, cylindrical to cylindric-clavate, pedicellate, bitunicate, fissitunicate (Fig. 16), with a shallow ocular chamber and faint ring (Figs. 15–16). Ascospores 45–52.5 × 22.5–27.5 µm (mean = 49 × 25 µm, n = 100), ellipsoid-fusiform, apices pointed, brown, guttulate, uniseptate, uniseriate or biseriate, slightly constricted at the septa, wall minutely verrucose, ascospore surrounded by a thick mucilaginous sheath, ends with a small subapical hood-like rim, and a long, appendage arising from both apices, up to 150 µm long and 5 µm diam. (Figs. 17–19).

Habitat: Freshwater. Saprobic on submerged wood (*Azadirachta indica* var. *siamensis*, *Erythrophleum teysmannii*, *Melaleuca cajuputi*, *Shorea obtusa*,



Figs. 12–19. *Jahnula appendiculata*. 12. Superficial ascoma with stalk on test block. 13. Squash mount of an ascoma with a long septate stalk. 14. Squash mount of asci and pseudoparaphyses. 15–16. Cylindric-clavate asci. 17–19. Ascospores with bipolar appendages and a thick mucilaginous sheath.

Measure bars for Fig. 12 = 500 µm. Fig. 13 = 100 µm. Fig. 14 = 50 µm. Figs. 15–18 = 40 µm. Fig. 19 = 20 µm.

S. roxburghii, Wrightia tomentosa, *Xylia xylocarpa*, *Zollingeria dongnaiensis*) in a peat swamp forest.

Geographical distribution: Thailand.

Specimens examined: **Thailand. Narathiwat:** Sirindhorn peat swamp forest on submerged test blocks (*M. cajuputi*), BIOTEC SS2414; BIOTEC SS2415; (*W. tomentosa*), BIOTEC SS2429; (*X. xylocarpa*), BIOTEC SS2438; (*A. indica*), BIOTEC SS2448; (*S. obtusa*), BIOTEC SS2466; 22 February 2003, Somsak Sivichai & Nattawut Boonyuen; (*E. teysmannii*), BIOTEC SS2900; (*X. xylocarpa*), BIOTEC SS2903; (*M. cajuputi*), BIOTEC SS2906; (*A. indica*), BIOTEC SS2911; BIOTEC SS2915; (*Z. dongnaiensis*), BIOTEC SS2922; (*S. roxbordhii*), BIOTEC SS2924; (*S. obtusa*), BIOTEC SS2934; 30 January 2004, Somsak Sivichai & Nattawut Boonyuen.

Comments: Morphological features of *J. appendiculata* from our study agree with the type collection reported by Pinruan et al. (2002). Ascospore size in this study agrees with the range that was reported for the type specimen, as did measurements for the ascomata and asci. All major characters also agreed with the holotype specimen. Pinruan et al. (2002) noted that the frequency of occurrence of *J. appendiculata* was 1.7% and regarded this species as a common fungus. In this study, seven of the nine timber species were colonized by *J. appendiculata* but the fungus did not occur on *E. teysmannii* and *S. siamensis*. *Jahnula appendiculata* is known only from one site and therefore may be well adapted to the acidic waters of the peat swamp forest. Moreover, it has not been collected on natural submerged wood and test blocks at other test sites (e.g. Khao Yai National Park, Doi Inthano National Park, Kaeng Krachan National Park, and Khao Sok National Park, Thailand, in the past eight years.

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